

## DECLARATION UNDER 37 C.F.R § 1.132

Sir:

I, Wayne Comper, hereby declare and say as follows:

1. That I am an Australian citizen , residing in Victoria, Australia, and am the inventor of the invention described and claimed in application Serial No. 09/892,797.

My curriculum vitae is attached as Exhibit A.

2. I have read and am familiar with the disclosure and pending claims of the above-captioned Application, as well as the issues raised in the pending final Office Action dated May 31, 2002.

3. I declare further that the following experiments were conducted at my direction and under my supervision and that the test results are true and correct to the best of my knowledge.

Two groups of rats were studied: I) untreated controls and ii) rats with experimental diabetes induced by administration of streptozotocin (STZ). Each of the rats was implanted with an osmotic pump containing radiolabelled IgG or transferrin. Steady state levels of radiolabelled protein in plasma were observed seven days after implantation of the osmotic pump. At that time collections were made of both urine and plasma. Estimates of the specific activity of protein in plasma were made by analysis of radioactivity and immuno-reactive material detected in the plasma. Immuno-unreactive (ghost) material in urine was estimated by the amount of radioactivity eluting at the position of the intact protein on a size exclusion column and specific activity of the protein in plasma.

The results for transferrin are set forth in the bar graph attached hereto. The amount of transferrin detected by immunoassay in both control animals and diabetic animals is about six times less than the amount of ghost transferrin detected. The amount of ghost transferrin estimated to be present in the urine of diabetic mice was significantly higher than in control rats, i.e., about a 47% increase in diabetic rats was observed. In contrast, the amount of intact transferrin detected by conventional radioimmune assay in the diabetic animals was not significantly different from that observed in the control animals.

The results obtained for IgG were similar to that of transferrin in that the amount of intact IgG detected by conventional radioimmune assay in control and diabetic animals was about the same. However, in both sets of animals, the amount of immuno-unreactive (ghost) material was significantly higher than the amount of intact IgG (at least about six times higher). The amount of ghost IgG was not significantly different in control and diabetic rats indicating that changes in IgG content in urine probably occur later than that observed for transferrin in diabetic animals.

### Conclusion


In my opinion, the test results demonstrate that:

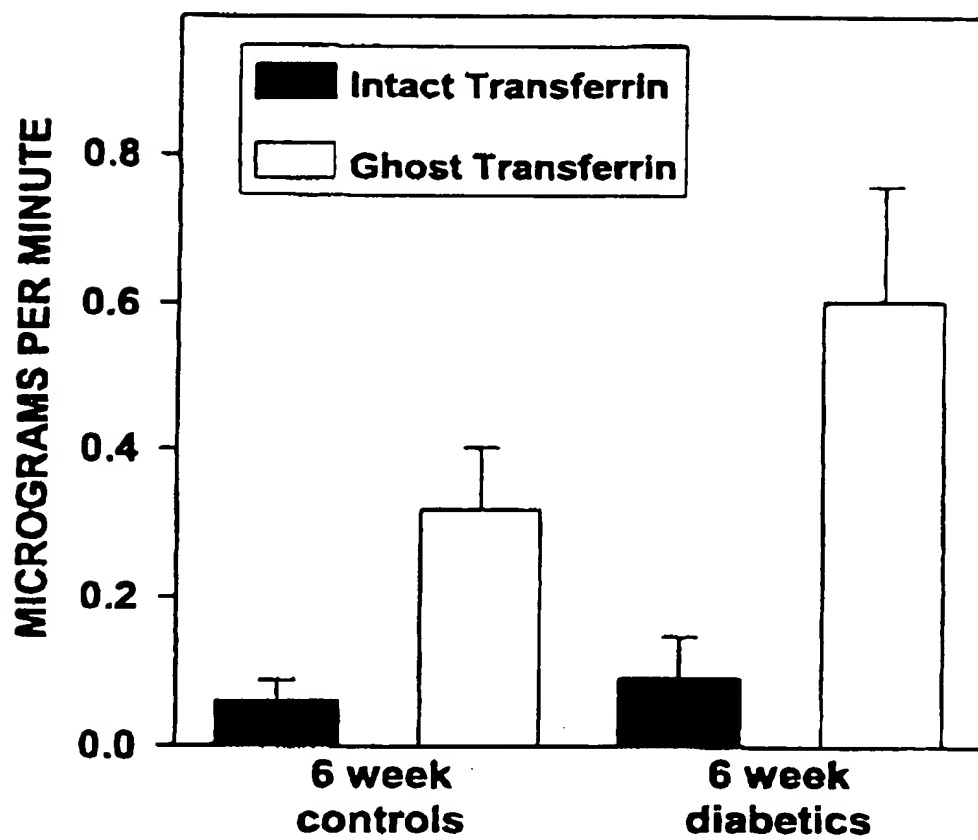
- (1) The majority of protein present in urine is not detectable by conventional immunoassay. As a result, conventional immunoassays are insufficient to detect renal complications of disease prior to the onset of kidney degeneration.

(2) Detection of immunoreactive and immuno-unreactive protein in a sample provides an accurate protein profile of the sample, which can be used to accurately diagnose renal complications of disease before the onset of kidney degeneration.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

11<sup>th</sup> September  
Date 2002

  
Wayne Comper



**CV of Wayne D. Comper**

**Present Position**  
Reader

**Professional Address**  
Department of Biochemistry and Molecular Biology,  
Monash University,  
Clayton, Victoria 3800,  
Australia.  
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**Degrees Held**  
B.Sc (Hons I), Monash University 1971  
PhD Monash University 1974  
DSc Monash University 1992

**Present Appointment**

1991  
Reader, Department of Biochemistry, Monash.

**Previous Appointments**

1974  
Postdoctoral Fellow, Northwestern University Medical School, Chicago, Illinois

1976  
Visiting Scientist, Dept Medical Physiology, Uppsala University, Uppsala, Sweden

1979  
Research Fellow, Dept Biochemistry, Monash University

1984  
Lecturer, Monash University

1988  
Visiting Professor, Nephrology Section, Rush Medical School, Chicago, Illinois

**Scientific Honours**  
Awarded the 1991 Silver Jubilee Prize for Research at Monash University

**Nationality**  
Australian

**Societies**  
American Society of Nephrology  
International Society of Nephrology  
Australian and New Zealand Society of Nephrology

**Invited reviewer of papers in journals**  
American Journal of Physiology  
Biophysical Chemistry  
Biophysical Journal

Biopolymers  
 Connective Tissue Research  
 Diabetes  
 European Journal of Physiology  
 Journal of American Society of Nephrology  
 Kidney International

#### Recent Invited Lectures

July 2001 International conference on 'Many faces of Osteoarthritis' Lake Tahoe USA  
 August 2000 Chicago USA Proteinuria: Reflector or initiator of renal dysfunction. Satellite meeting to the International Society of Hypertension.  
 October 1999 Montecatini Terme, Italy. Kidney, proteins and growth factors.  
 During 1990-99 invited to give over 20 lectures at international conferences including three Gordon conferences.

#### Postgraduate Supervision

Current 5 PhD students and 2 Honours students.  
 During 1990 -2000 12 PhD students have graduated from the lab.

#### Collaborations

Dr G. Bakris, Hypertension Unit, Rush Medical School, Chicago, Illinois  
 Dr A. Singh, Hektoen Institute, Chicago, Illinois  
 Dr R. Nelson, NIH, Phoenix, Arizona  
 Dr D. Nikolic-Paterson, Prof. R. Atkins, Nephrology, Monash Medical Centre  
 Dr G. Jerums, Endocrinology Unit, Austin Hospital, Melbourne

## LIST OF PUBLICATIONS

### Thesis

- I. Comper, W.D. (1974) The biological function of proteoglycans. Ph.D. thesis, Monash University, Australia. 200pp
- II. Comper, W.D. (1991) Structure and function of extracellular matrices of connective tissues. D.Sc. thesis, Monash University, Australia.

### Books/Editorships

1. Comper, W.D. (1981) "Heparin (and related polysaccharides). Structural and Functional Properties", Gordon & Breach Science Publishers, London & New York, 265 pp.
2. Comper, W.D.(ed.) (1996) 'Extracellular Matrix. Volume 1. Tissue Function'. Harwood Academic Press, Amsterdam, The Netherlands, 482pp.
3. Comper, W.D.(ed.) (1996) 'Extracellular Matrix. Volume 2. Molecular Components and Interactions'. Harwood Academic Press, Amsterdam, The Netherlands, 376pp.

### Chapters in books

4. Comper, W.D. and Wik, K-O. (1979) Diffusion of hyaluronate in concentrated solutions. *Acta Universitatis Upsaliensis* 334, 25 pp.
5. Preston, B.N., Laurent, T.C. and Comper, W.D. (1984). "Transport of molecules in connective tissue polysaccharide solutions" in 'Molecular Biophysics of the Extracellular Matrix' (Amott, S., Rees, D.A. and Morris, E.R. eds) Humana Press, Clifton, New Jersey, pp 119-162.
6. Comper, W.D. (1984) "Interstitium" in 'Edema: Basic Science and Clinical Manifestations. A comprehensive treatise.' (Staub, N. and Taylor, A. eds) Raven Press, New York, pp 229-262.
7. Comper, W.D. (1990) Osmotic and hydraulic flows in proteoglycan solutions in Biomechanics of Diarthrodial Joints (Mow, V.C., Ratcliffe, A. and Woo, S.L.Y. eds), Springer-Verlag, New York, pp 345-362.
8. Comper, W.D. (1991) Physicochemical aspects of cartilage extracellular matrix in 'Cartilage Molecular Aspects' (Hall, B.K. and Newman, S.A. eds). Telford Press, New Hampshire pp 59-96.
9. Comper, W.D. and Williams, R.P.W. (1992) Mechanism of osmotic flow in Mechanics of Swelling: From Clays of Living Cells and Tissues

(Karalis, T.K., ed.) NATO ASI Series H, Springer-Verlag, Berlin, 64, 743-762

10. Comper, W.D.(1992) Diffusion-convection in biopolymer solutions in *Dynamical Phenomena at Interfaces, Surfaces and Membranes* (Beysens,D., Boccara, N. and Forgacs, G. eds) Nova Sci. NY. pp 521-542.
11. Comper, W.D. and Zamparo, O. (1992) Sedimentation analysis of proteoglycans in *Analytical Ultracentrifugation in Biochemistry and Polymer Science*. (Harding, S.E., Rowe, A.J. and Horton, J.C. eds), Royal Society of Chemistry, Redwood Press, England, pp 532-548.
12. Comper, W.D. and Preston, B. N. (1992) Analytical ultracentrifuge as a tool for diffusion measurements. Cross diffusion effects in ternary polymer:polymer:solvent systems in *Analytical Ultracentrifugation in Biochemistry and Polymer Science*. (Harding, S.E., Rowe, A.J. and Horton, J.C. eds), Royal Society of Chemistry, Redwood Press, England pp 428-442.
13. Comper, W.D. (1995) Evolution and development of extracellular matrices is correlated with the sulfation of matrix macromolecules to form highly variable macroion binding templates in *Interplay of genetic and physical processes in the development of biological form* (Beysens, D., Felix, M-A., Forgacs, G. and Gaill, F. eds.) Les Houches Workshop. World Scientific, Singapore, pp121-127.
14. Comper, W.D. (1996) Water. Dynamic aspects in Comper, W.D.(ed.) 'Extracellular Matrix. Volume 2. Molecular Components and Interactions'. Harwood Academic Press, Amsterdam, The Netherlands, pp1-21.
15. Comper, W.D., Glasgow, E.F. and Singh, A.K. (1996) The Glomerulus in Comper, W.D.(ed.) 'Extracellular Matrix. Volume 1. Tissue Function'. Harwood Academic Press, Amsterdam, The Netherlands, pp409-441.
16. Comper, W.D., Burne, M.J., Osicka, T.M., Pratt, L.M., Smit, M.F. and Vyas, S.V. (1997) New insights into the renal passage of albumin. in *Connective Tissue Biology; Integration and Reductionism*, Reed, R. and Rubin, K. eds., Portland Press, London , pp253-268.

#### Refereed journal Reviews

17. Comper, W.D. and Laurent, T.C. (1978) Physiological function of connective tissue polysaccharides. *Physiol. Rev.* 58, 255-315.
18. Laurent, T.C., Preston, B.N., Comper, W.D. and Sundelof, L-O. (1981). "The interactions in concentrated polysaccharide solutions" in *Gums and Stabilisers for the Food Industry* (Phillips, G.O., Wedlock, D.J. and



Williams, P.A. eds) Pergamon Press (also as (1982) Prog. Food 6, 69-76).

19. Comper, W.D. (1982) Collisions in coffee. Chaos in solution. Hemisphere 26, 376-379.
20. Comper, W.D. and Preston, B.N. (1984). Rapid polymer transport in concentrated solutions. Adv. Polym. Sci. 55, 105-151.
21. Comper, W.D. and Williams, R.P.W. (1987) Dynamics of polysaccharide solutions in Interstitial - Lymphatic Liquid and Solute Movement (Staub, N.C., J.C. Hogg and A.R. Hargens eds.) Adv. in Microcirculation 13, 43-56.
22. Comper, W.D., Williams, R.P.W. and Zamparo, O. (1990). An invited review. Water transport in extracellular matrices. Connective Tissue Research 25, 3-16.
23. Comper, W.D. and Glasgow E.F. (1995) Charge selectivity in kidney ultrafiltration. An editorial review. Kidney Int. 47, 1242-1251.

#### Original refereed research papers

24. Meyer, F., Comper, W.D. and Preston, B.N. (1971) Model connective tissue systems. A physical study of gelatin gels containing proteoglycan. Biopolymers 10, 1351-1364.
25. Comper, W.D. and Preston, B.N. (1974) Model connective tissue systems. A study of polyion-mobile ion and of excluded volume interactions of proteoglycans. Biochem. J. 143, 1-9.
26. Comper, W.D. and Preston, B.N. (1975) Model connective tissue systems. Measurement of ion flux across gel membranes containing proteoglycans. J. Coll. & Int. Sci. 53, 379-390.
27. Comper, W.D. and Preston, B.N. (1975) Model connective tissue systems. Membrane phenomena of gel membranes containing proteoglycans and other polyelectrolytes. J. Coll. & Int. Sci. 53, 391-401.
28. Comper, W.D., Lisberg, W. and Veis, A. (1976) Diffusion potentials of polyelectrolytes and their possible relationship to biological electrochemical phenomena. J. Coll. & Int. Sci. 57, 345-352.
29. Comper, W.D. (1977) Electric potentials generated by connective tissue polysaccharides in glass capillaries. Biochim. Biophys. Acta 497, 816-819.

30. Comper, W.D. and Veis, A. (1977) The mechanism of nucleation for *in vitro* collagen fibril formation. *Biopolymers* **16**, 2113-2131.
31. Comper, W.D. and Veis, A. (1977) Characterization of nuclei in *in vitro* collagen fibril formation. *Biopolymers* **16**, 2133-2142.
32. Comper, W.D. and Laurent, T.C. (1978) On the interaction of dextran and albumin. An estimate of the enthalpic contribution. *Biochem. J.* **175**, 703-708.
33. Preston, B.N., Laurent, T.C., Comper, W.D. and Checkley, G.J. (1980) Rapid polymer transport in concentrated solutions through the formation of ordered structures. *Nature* **287**, 499-503.
34. Comper, W.D. and Preston, B.N. (1981) On the spontaneous self-organisation of various biopolymer systems to form structured flows. *Biochem. Int.* **3**, 558-564.
35. Comper, W.D., DeWitt, M. and Lowther, D. (1981) Effects of anti-inflammatory drugs on proteoglycan degradation as studied in rabbit articular cartilage in organ culture. *Biochem. Pharmacol.* **30**, 459-468.
36. Preston, B.N., Comper, W.D., Hughes, A.E., Snook, I. and Van Megan, W. (1982) Diffusion of dextran at intermediate concentrations. *J. Chem. Soc., Faraday Trans. I.* **78**, 1209-1221.
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42. Preston, B.N., Comper, W.D., Checkley, G.J. and Kitchen, R.G. (1983). Kinetics of multicomponent transport by structured flow in polymer solutions. 3. Effects of varying conditions and molecular components. *J. Phys. Chem.* 87, 662-667.
43. Comper, W.D., Preston, B.N., Laurent, T.C., Checkley, G.J. and Murphy, W.H. (1983) Kinetics of multicomponent transport by structured flow in polymer solutions. 4. Relationships between the formation of structured flows and kinetics of polymer transport. *J. Phys. Chem.* 87, 667-673.
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45. Comper, W.D., Checkley, G.J. and Preston, B.N. (1984) Kinetics of multicomponent transport by structured flow in polymer solutions 5. Ternary diffusion in the system dextran-poly(vinylpyrrolidone)-water. *J. Phys. Chem.* 88, 1068-1076.
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47. Comper, W.D. and Preston, B.N. (1984) Molecular parameters of polymers affecting the stability of interdiffusing fluid layers. *J. Coll. Int. Sci.* 99, 305-314.
48. Comper, W.D., MacDonald, P., Preston, B.N. (1984) Cross diffusion between albumin and dextran. *J. Phys. Chem.* 88, 6031-5036.
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50. Daivis, P., Snook, I., Van Megan, W., Preston, B.N. and Comper, W.D. (1984) Dynamic light scattering measurements of diffusion in polymer-polymer-solvent systems. *Macromolecules* 17, 2376-2380.
51. Comper, W.D., Van Damme, M-P. and Preston, B.N. (1985) Kinetics of multicomponent transport by structured flow in polymer solutions. 7. Polysaccharide-saccharide system. *J. Phys. Chem.* 89, 128-134.
52. Comper, W.D., Checkley, G.J. and Preston, B.N. (1985) Kinetics of multicomponent transport by structured flow in polymer solutions. 8. Further studies on poly(vinylpyrrolidone) and dextran system. *J. Phys. Chem.* 89, 1551-1555.
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54. Comper, W.D., Preston, B.N. and Davis, P. (1986) The approach of dextran mutual diffusion coefficients to molecular weight independence in semi dilute solutions of polydisperse dextran fractions. *J Phys. Chem.* **90**, 128-132.
55. Comper, W.D., Pratt, L. and Handley, C.J. (1987) Cell transport in model extracellular matrices. *Arch. Biochem.* **252**, 60-70.
56. Comper, W.D., Williams, R.P.W., Checkley, G.J. and Preston, B.N. (1987) Stability analysis in diffusion-convection systems with and without cross diffusion. *J. Phys. Chem.* **91**, 993-1000.
57. Williams, R.P.W. and Comper, W.D. (1987) Osmotic flow caused by non-ideal macromolecular solutes. *J. Phys. Chem.* **91**, 3443-3448.
58. Comper, W.D. and Williams, R.P.W. (1987) Hydrodynamics of concentrated proteoglycan solutions. *J. Biol. Chem.* **262**, 13464-13471.
59. Ghosh, S. and Comper, W.D. (1988) Oriented fibrillogenesis of collagen *in vitro* by ordered convection. *Conn. Tiss. Res.* **17**, 33-41.
60. Bolis, S., Handley, C.J. and Comper, W.D. (1989). Passive loss of proteoglycan from articular cartilage explants. *Biochim. Biophys. Acta* **993**, 157-167.
61. Comper, W.D. and Zamparo, O. (1989) Hydraulic conductivity of polymer matrices. *Biophys. Chem.* **34**, 127-135.
62. Van Damme, M.P., Murphy, W.H., Comper, W.D., Preston, B.N. and Winzor D.J. (1989). Evaluation of non-ideality from gel chromatography partition coefficients by gel chromatography - a technique with greater versatility than equilibrium dialysis. *Biophys. Chem.* **33**, 115-125.
63. Vassiliou, P., Tay, M. and Comper, W.D. (1989). Partial ischemia and proteinuria during isolated kidney perfusion is accompanied by the release of vascular [<sup>35</sup>S]heparan sulfate. *Biochem. Int.* **19**, 1241-1251.
64. Zamparo, O. and Comper, W.D., (1989). Hydraulic conductivity of chondroitin sulfate proteoglycan solutions. *Arch. Biochem. Biophys.* **274**, 259-269.
65. Comper, W.D. (1990) Extracellular matrix interactions: Sulfation of connective tissue polysaccharides creates macroion binding templates and conditions for dissipative structure formation. *J. Theor. Biol.* **145**, 497-509.

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67. Comper, W.D. and Zamparo, O. (1990) The hydrodynamic properties of connective tissue polysaccharides. *Biochem. J.* **269**, 561-564.
68. Newman, S.A. and Comper, W.D. (1990) Generic mechanisms for morphogenesis and pattern formation development. *Development* **110**, 1-26.
69. Tay, M., Comper, W.D. Vassiliou, P., Glasgow, E.F. Baker M.S. and Pratt, L. (1990). The inhibitory action of oxygen radical scavengers on proteinuria and glomerular heparan sulfate loss in the isolated perfused kidney. *Biochem. Int.* **20**, 767-778.
70. Williams, R.P.W. and Comper, W.D. (1990) Osmotic flow caused by polyelectrolytes. *Biophys. Chem.* **36**, 223-231.
71. Williams, R.P.W. and Comper, W.D. (1990) Appendix. The analogy between sedimentation equilibrium in the ultracentrifuge and osmotic water flow across membranes. *Biophys. Chem.* **36**, 231-234.
72. Zamparo, O. and Comper, W.D. (1990) Model anionic polysaccharide matrices exhibit lower charge selectivity than is normally associated with kidney ultrafiltration. *Biophys. Chem.* **38**, 167-178.
73. Tay, M., Comper, W.D., and Singh, A.K. (1991) Charge selectivity in kidney ultrafiltration is associated with glomerular uptake of transport probes. *Am. J. Physiol.* **260**, F549-F554.
74. Zamparo, O. and Comper, W.D. (1991) The hydrodynamic frictional coefficient of polysaccharides: the role of the glycosidic linkage. *Carbohydr. Res.* **212**, 193-200.
75. Comper, W.D. and Lyons, K.C. (1993) Non electrostatic factors govern the hydrodynamical properties of articular cartilage proteoglycan. *Biochem. J.* **289**, 543-547.
76. Comper, W.D., Tay, M., Lee, A.S.N. and Adal, Y (1993) Anionic charge concentration of rat kidney glomeruli and glomerular basement membrane. *Biochem. J.* **289**, 647-652.
77. Wills, P., Comper, W.D. and Winzor, D.J. (1993) Thermodynamic nonideality in macromolecular solutions: Interpretation of virial coefficients. *Arch. Biochem. Biophys.* **300**, 206-212.
78. Lyons, K. and Comper, W.D. (1993) Diffusion of tritiated water in

chondroitin sulfate solutions. Biophys. Chem. 47, 61-66.

79. Adal, Y., Pratt, L.M. and Comper, W.D. (1994) Transglomerular transport of DEAE-dextran in the isolated perfused kidney. Microcirculation 1, 169-174.
80. Comper, W.D. (1994) The thermodynamic and hydrodynamic properties of macromolecules that influence the hydrodynamics of porous systems. J. Theor. Biol. 168, 421-427.
81. Comper, W.D., Tay, M., Wells, X. and Dawes, J. (1994) Desulphation of dextran sulphate during kidney ultrafiltration. Biochem. J. 297, 31-34.
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86. Vyas, S.V., Parker, J-A and Comper, W.D. (1995) The uptake of dextran sulphate by glomerular intracellular vesicles during kidney ultrafiltration. Kidney Int. 47, 945-950.
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Competition between polyanions in glomerular binding and renal clearance. *Arch Biochem. Biophys.* **340**, 257-264.

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99. Burne, M.J., Osicka, T.M. and Comper, W.D. (1999) Fractional clearance of high molecular weight proteins in conscious rats using a continuous infusion method. *Kidney Int* **55**, 261-270.
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101. Osicka, T.M., Hankin, A.R. and Comper, W.D. (1999) Puromycin aminonucleoside nephrosis results in a marked increase in fractional clearance of albumin. *Am J. Physiol.* **277**, F139-F145.
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